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Pancreatic Cancer Stroma: Friend or Foe?

Jesse Gore[1] and Murray Korc[1,*]
[1]Departments of Medicine and Biochemistry and Molecular Biology, Indiana University School of Medicine, Melvin and Bren Simon Cancer Center and Pancreatic Cancer Signature Center, Indianapolis, IN 46202, USA
*Correspondence: mkorc@iu.edu
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Pancreatic cancer desmoplasia is thought to confer biological aggressiveness. In this issue of *Cancer Cell*, Özdemir and colleagues and Rhim and colleagues demonstrate that targeting the stroma results in undifferentiated, aggressive pancreatic cancer that responds to checkpoint blockade or antiangiogenic therapy, uncovering a protective role by stroma in this cancer.

Pancreatic ductal adenocarcinoma (PDAC) has a dismal 5-year survival rate of 6% and is projected to be the second leading cause of cancer death by 2030 (Rahib et al., 2014). PDACs harbor high-frequency mutations in major cancer driver genes, including *KRAS* (95%), *CDKN2A* (~95% mutated and ~5% epigenetically silenced), *TP53* (75%), and *SMAD4* (55%), numerous low-frequency driver mutations, and regions of hypermutation termed kataegis (Alexandrov et al., 2013). There is also overexpression of growth factors, their high affinity tyrosine kinase receptors, TGF-β isoforms, and sonic hedgehog (Shh), as well as loss of negative growth constraints (Whipple and Korc, 2008).

PDACs exhibit desmoplasia, which derive from pancreatic stellate cells that are activated to proliferate and produce collagens, laminin, and fibronectin (Apte et al., 2013). Consequently, the tumor microenvironment exhibits enhanced stiffness (elastic modulus), increased hyaluronic acid content, and elevated hydrostatic pressures that may blunt effective intratumoral drug delivery (Provenzano et al., 2012). The stroma is also believed to contribute to tumor hypoperfusion and hypoxia and harbor infiltrative macrophages and inflammatory cells with the potential to suppress cancer-directed immune mechanisms. In theory, therefore, stroma depletion could enhance drug delivery to the cancer cells within the tumor mass while disrupting deleterious stroma-cancer cell interactions.

Genetically engineered mouse models (GEMMs) of PDAC have transformed our understanding of PDAC pathobiology, yielding novel information regarding potential therapeutic targets (Guerra and Barbacid, 2013). These autochthonous models recapitulate events occurring in human PDAC, including pancreatic intraepithelial neoplasia (PanIN), progression to murine PDAC (mPDAC), acinar-to-ductal metaplasia (ADM), abundant stroma, inflammatory changes, and metastasis in the context of an intact immune system. Despite its success in GEMMs, stroma-targeting clinical trials have failed. In this issue of *Cancer Cell*, Özdemir et al. (2014) and Rhim et al. (2014) used two distinct strategies to address the root cause for this failure.

Özdemir et al. (2014) targeted αSMA$^+$ myofibroblasts by crossing *Ptfla*$^{cre/+}$; *Kras*$^{LSL\text{-}G12D/+}$;*Tgfbr2*$^{flox/flox}$ (KTC) mice with αSMA-tk transgenic mice and administering ganciclovir (GCV) to the resultant KTC;αSMA-tk mice at PanIN (early) or mPDAC (late) stages. Remarkably, myofibroblast depletion at either stage yielded undifferentiated and invasive tumors with necrotic regions; both groups succumbed earlier than mice not receiving GCV. Similar results were obtained in *Pdx1*$^{cre/+}$;*Kras*$^{LSL\text{-}G12D/+}$; *Trp53*$^{R172H/+}$ (KPC) mice crossed with αSMA-tk mice, confirming that desmoplasia protects the host.

Özdemir et al. (2014) also showed that key concepts regarding the deleterious effects of stroma in PDAC need to be revamped. Thus, myofibroblast depletion led to decreased elastic modulus without improving gemcitabine's therapeutic actions or altering hyaluronic acid content, as determined by assaying for its marker hyaluronic acid binding protein. Crossing KTC mice with Cre-reporter mice and depleting αSMA$^+$ cells revealed that reporter-positive cancer cells exhibited increased epithelial-to-mesenchymal transition (EMT), elevated expression of pro-EMT transcription factors (*Twist, Snail,* and *Slug*), a stem cell-like phenotype (with increased CD44$^+$CD133$^+$ cell populations), in vitro sphere formation, and in vivo tumorigenicity. Myofibroblast depletion at either the PanIN or mPDAC stage attenuated tumor angiogenesis without altering glycolysis and enhanced tumor hypoxia, which was determined by staining for pimonidazole adduct formation. Moreover, expression profiling revealed that CAFs exhibited ECM remodeling and a proangiogenic profile in contrast to normal pancreas fibroblasts.

Myofibroblast depletion was also associated with altered immune gene expression and changed infiltrating immune cell populations, specifically decreased CD4$^+$ effector T cells, increased CD4$^+$Foxp3$^+$ regulatory T cells (Tregs), and decreased cytotoxic CD8$^+$/Treg and CD3$^+$/CD11b$^+$ ratios. Importantly, *Ctla4* expression was increased. Treatment of myofibroblast-depleted mice with a CLTA-4 blocking antibody attenuated PDAC progression, improved overall survival, induced tumor clearance in up to 25% of the pancreas, and reprogrammed the transcriptome to a pattern that resembled control (myofibroblast-competent) tumors.

Rhim et al. (2014) chose to delete *Shh* in the cancer cells to suppress mPDAC stroma. Accordingly, they crossed *Pdx1-Cre;Kras*$^{LSL\text{-}G12D/+}$;*p53*$^{fl/+}$; *Rosa26* $^{LSL\text{-}YFP/+}$ (KP$^{fl/+}$CY) mice with *Shh*$^{fl/fl}$ mice, generating ShhKP$^{fl/+}$CY mice. These mice exhibited decreased Gli1 expression and stroma formation. Although *Shh* deletion per se did not alter pancreatic development, by comparison with KP$^{fl/+}$CY mice, ShhKP$^{fl/+}$CY mice had more frequent PanIN and ADM lesions at a young age, an earlier appearance of mPDAC that was more undifferentiated, and increased metastasis; the

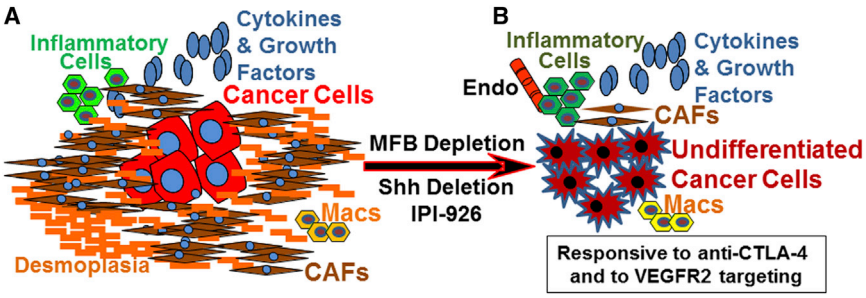

Figure 1. Deleterious Consequences of Targeting the Stroma in Pancreatic Cancer

(A) Highly desmoplastic PDAC. These tumors exhibit activated cancer-associated fibroblasts (CAFs) that synthesize and release collagens, laminin, and fibronectin (desmoplasia); are infiltrated by various inflammatory cells including macrophages (Macs); and produce excess growth factors and cytokines. Cancer cells in these lesions may be well, moderately, or poorly differentiated.

(B) Stroma-depleted PDAC. Engineered myofibroblast (MFB) depletion, genetic deletion of sonic hedgehog (Shh), or Smoothened receptor targeting with IPI-926 were used to dramatically decrease the presence of MFBs and CAFs in several genetically-engineered mouse models (GEMMs) of PDAC. Each of these strategies yielded undifferentiated PDAC, enhanced EMT, increased pancreatic cancer cell proliferation, and altered immune cell infiltrate profiles. Direct MFB depletion was associated with attenuated angiogenesis and an increased response to immune checkpoint blockade with an anti-CTLA-4 antibody. Shh deletion or IPI-296 treatment was associated with increased angiogenesis (Endo) and metastasis, a greater incidence of cachexia, and increased responsiveness to DC101, which targets VEGFR2. Thus, in several GEMMs and with different strategies, targeting the stroma unmasks a previously unrecognized protective effect in PDAC.

mice also died more rapidly. In this context, pancreatic cancer cells (PCCs) showed enhanced proliferation and angiogenesis, increased Zeb1 and Slug expression consistent with EMT, and reduced $CD45^+$ myeloid cells and $F4/80^+$ monocytes infiltration. Similar results were observed when KPC mice were treated with IPI-926, a Smoothened inhibitor. Thus, genetic *Shh* deletion or pharmacological targeting of Shh signaling pathways attenuates stroma formation and leads to more aggressive mPDAC.

KPC mice typically die due to locally invasive disease, extensive metastases, or marked cachexia. This course was not altered by gemcitabine treatment and was worsened by added treatment with IPI-926, most notably because of faster onset of severe cachexia, which also occurred in a subset of control $ShhKP^{fl/+}$CY mice that were not treated with IPI-926. PanIN and ADM lesions were also increased by IPI-926 treatment, and this effect was partially prevented by gemcitabine treatment. These findings suggest that the actions of IPI-296 in promoting cachexia are due to its effects on mPDAC aggressiveness and that gemcitabine may be effective at attenuating precursor lesion progression to PDAC. Given that patients with PDAC often develop cachexia (Fearon et al., 2011), it will be important to determine whether cachexia correlates with attenuated stroma and enhanced tumor angiogenesis and to delineate the mechanisms whereby stroma protects against cachexia.

Rhim et al. (2014) proposed that mesenchymal stromal cells exert an antiangiogenic effect on endothelial cells, which contrasts with the finding by Özdemir et al. (2014) that CAFs express proangiogenic factors. Rhim et al. (2014) also targeted VEGFR2 signaling with the blocking antibody DC101, which prolonged the survival of $ShhKP^{fl/+}$CY mice, but not $KP^{fl/+}$CY mice, even though mPDAC VEGF expression was similar in both groups. The authors suggest that stroma depletion leads to greater dependence on VEGF rather than its further induction and demonstrates that undifferentiated PDAC in patients is associated with enhanced tumor angiogenesis and decreased stroma.

Taken together (Figure 1), these two paradigm-changing studies provide important new insights on PDAC pathobiology: (1) the stroma in PDAC is protective, and this protective action is potentially already exerted at the PanIN stage; (2) targeting the stroma can lead to a more biologically aggressive form of PDAC with enhanced PCC proliferation, but can also "prime" the tumor to more efficiently respond to checkpoint blockade and to antiangiogenic therapy; and (3) while many aspects of stromal biology are still valid, it is important to reassess various therapeutic approaches in light of the current findings, and more specifically target the cancer cells and not the stromal cells while bearing in mind that the actions of the stroma in PDAC may be context dependent. Although the concepts of a protective stroma and targeting angiogenesis in PDAC are not novel, we now have a better understanding of their potential roles, which may allow for improved precision therapy. Moreover, given the complexity and plasticity of the stroma and associated immune cells, further studies are necessary to more clearly delineate deleterious and beneficial aspects of stroma biology in PDAC. Finally, because oncogenic Kras may induce PanIN-associated stroma, we also need to study the pathways that allow PanIN and ultimately PCCs to bypass the protective effects of the stroma.

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